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A New Species of *Tylototriton* from Northern Vietnam (Amphibia: Urodela: Salamandridae)

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Abstract: A new species of the salamandrid genus *Tylototriton* is described from Ha Giang and Cao Bang provinces, northern Vietnam, based on molecular and morphological data. The new species differs morphologically from all known congeners in the combination of blackish body coloration; medium-sized body; distinctly rough skin; tubercular vertebral ridge; knob-like rib nodules; large eye; and low, narrow tail. The distribution pattern of species of *Tylototriton* in Vietnam is briefly discussed.

Key words: *Tylototriton*; Molecular phylogeny; Morphology; New species; Vietnam

INTRODUCTION

The taxonomic status of a species of *Tylototriton* from Ha Giang and Cao Bang provinces, northern Vietnam has been controversial. The species has been identified as *T. asperrimus*, *T. vietnamensis*, or *Tylototriton* cf. *vietnamensis* after it was first reported as *T. vietnamensis* (Böhme et al., 2005; Stuart et al., 2010; Yuan et al., 2011). Recent phylogenetic analyses showed that the populations of this *Tylototriton* form a lineage divergent from the other congeners with substantial genetic differentiations in both mitochondrial and nuclear DNA sequences (Weisrock et al., 2006; Stuart et al., 2010; Yuan et al., 2011; Gu et al., 2012; Shen et al., 2012). However, none of aforementioned authors has succeeded in clarifying the taxonomic status of this species because they only analyzed DNA sequences deposited in GenBank but did not possess their own morphological data of the species and/or topotypic samples of the related congeners for both genetic and morphological comparisons. It is clear that the species phylogenetically belongs to the species group with a blackish body (the *Tylototriton asperrimus* group [Fei et al., 2005] or subgenus *Yaotriton* Dubois et Raffaëlli, 2009), which includes *T. asperrimus* Unterstein, 1930, *T. broadoridgus* Shen, Jiang et Mo, 2012, *T. hainanensis* Fei, Ye et Yang, 1984, *T. lizhenchangi* Hou, Zhang, Jiang, Li et Lu, 2012, *T. notialis* Stuart, Phimmachak, Sivongxay et Robichaud, 2010, *T. vietnamensis* Böhme, Schöttler, Nguyen et Köhler, 2005, and *T. wenxianensis* Fei, Ye et Yang, 1984 (after Hou et al. [2012]). In this study, we examine the taxonomic status of a *Tylototriton* sp. from northern Vietnam using both genetic and morphological data.

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Materials and Methods

Molecular phylogenetic analyses

We obtained sequence data of NADH dehydrogenase subunit 2 region (ND2) of mitochondrial DNA (mtDNA) from liver tissue samples preserved in 99% ethanol. Methods for DNA extraction and amplification and sequencing of the DNA fragment are the same as reported by Nishikawa et al. (2011) with a minor modification. The PCR primers were Sal_ND2_F1 (forward: 5'-AAGCTTTTGGGCCCATACC-3') and Sal_ND2_R2 (reverse: 5'-GGTTGCATTCAGAAGATGTG-3'), and the cycle sequencing primers, Sal_ND2_R1 (reverse: 5'-GTTATAAATATGGAKGARGTTA-3') and Sal_ND2_F2 (forward: 5'-ATAGCATAYTCRTCYATTGC-3'), were designed in the middle of the ND2 region, in addition to the two PCR primers shown above. Prior to the phylogenetic analyses, we plotted the uncorrected sequence divergences (p-distance) against the number of transitions and transversions to test for saturation in substitutions within the ingroup samples of *Tylototriton*. We confirmed that ND2 does not show any signs of saturation (data not shown).

We reconstructed phylogenetic trees using our own samples of 16 specimens of *Tylototriton* and a specimen of *Echinotriton andersoni*. For comparison, DNA sequences of 17 specimens of *Tylototriton* and one specimen each of *E. chinhaiensis*, *Pleurodeles waltl*, and *Notophthalmus viridescens* were obtained from GenBank (Table 1, Fig. 1). Outgroup species (Sample number 31–37 in Table 1) were selected based on observations of Weisrock et al. (2006).

The optimum substitution models were selected by Kakusan4 (Tanabe, 2011). We then constructed phylogenetic trees by Bayesian inference (BI) and maximum likelihood (ML) methods using MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001) and TREEFINDER ver. Mar. 2011 (Jobb, 2011), respectively. The criterion used for model selection was AIC.

The best substitution models for BI and ML were the general time reversible model (GTR: Tavaré, 1986)+G and the transition model (TIM: Jobb, 2011) with gamma shape parameter (G), respectively. Two independent runs of four Markov chains were conducted for ten million generations. We sampled one tree every 100 generations and calculated a consensus topology for 30,001 trees after discarding the first 30,000 trees (burn-in=3,000,000).

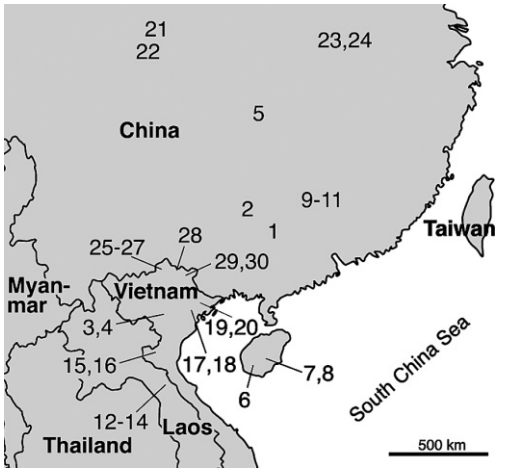

FIG. 1. Map showing localities of ingroup samples used for molecular analyses. For locality numbers, refer to Table 1.

For the Bayesian analysis, we considered posterior probabilities (bpp) 95% or greater as significant support (Leaché and Reeder, 2002). The robustness of the ML tree was tested using bootstrap analysis (Felsenstein, 1985) with 1000 replicates. We regarded tree topologies with bootstrap values (bs) of 70% or greater as sufficiently supported (Huelsenbeck and Hillis, 1993).

Morphological comparisons

We compared morphology of adult male specimens of *Tylototriton* sp. from northern Vietnam and all species of the *Tylototriton asperrimus* group except for *T. broadoridgus*. These specimens were collected from 10 localities covering the whole distribution range of the group, including topotypic specimens of *T. asperrimus* and *T. lizhenchangi* and speci-

TABLE 1. Specimens for molecular analyses. CIB=Chengdu Institute of Biology; FMNH=Field Museum of Natural History; HNNU=Henan Normal University; IEBR=Institute of of Ecology and Biological Resources; KUHE=Graduate School of Human and Environmental Studies, Kyoto University; MVZ=Museum of Vertebrate Zoology, University of California, Berkeley; ROM=Royal Ontario Museum; VNMN=Vietnam National Museum of Nature.

Sample no.	Species or subspecies	Voucher	Locality	GenBank accession no.
Ingroup				
1	*Tylototriton asperrimus*	CIB 200807055	Jinxiu, Guangxi, China	KC147815
2	*Tylototriton asperrimus*	CIB 70063	Longsheng, Guangxi, China	KC147816
3	*Tylototriton asperrimus*	VNMN TAO1213	Thuong Tien, Hoa Binh, Vietnam	AB769530
4	*Tylototriton asperrimus*	VNMN TAO1214	Thuong Tien, Hoa Binh, Vietnam	AB769531
5	*T. broadoridgus*	CIB 200085	Sangzhi, Hunan, China	KC147814
6	*T. hainanensis*	MVZ 230352	Jianfengling, Hainan, China	DQ517850
7	*T. hainanensis*	CIB 20081048	Diaoluoshan, Hainan, China	KC147817
8	*T. hainanensis*	CIB 20081049	Diaoluoshan, Hainan, China	KC147818
9	*T. lizhenchangi*	KUHE 42316	Yizhang, Hunan, China	AB769532
10	*T. lizhenchangi*	KUHE 42317	Yizhang, Hunan, China	AB769533
11	*T. lizhenchangi*	KUHE 46347	Yizhang, Hunan, China	AB769534
12	*T. notialis*	FMNH 271120	Boualapha, Khammouan, Laos	HM462061
13	*T. notialis*	FMNH 271121	Boualapha, Khammouan, Laos	HM462062
14	*T. notialis*	FMNH 271122	Boualapha, Khammouan, Laos	HM462063
15	*T. notialis*	VNMN TAO1229	Pu Hoat, Nghe An, Vietnam	AB769535
16	*T. notialis*	VNMN TAO1235	Pu Hoat, Nghe An, Vietnam	AB769536
17	*T. vietnamensis*	KUHE 55171	Yen Tu, Bac Giang, Vietnam	AB769537
18	*T. vietnamensis*	KUHE 55172	Yen Tu, Bac Giang, Vietnam	AB769538
19	*T. vietnamensis*	IEBR 3243	Son Dong, Bac Giang, Vietnam	HM770088
20	*T. vietnamensis*	IEBR 3244	Son Dong, Bac Giang, Vietnam	HM770089
21	*T. wenxianensis wenxianensis*	CIB 20090527	Wenxian, Gansu, China	KC147813
22	*T. w. wenxianensis*	MVZ 236632	Pingwu, Sichuan, China	DQ517855
23	*T. w. dabienicus*	HNNU 1004II015	Shangcheng, Anhui, China	KC147811
24	*T. w. dabienicus*	HNNU 1004II024	Shangcheng, Anhui, China	KC147812
25	*Tylototriton* sp.	VNMN 3390	Quan Ba, Ha Giang, Vietnam	AB769539
26	*Tylototriton* sp.	KUHE 55077	Quan Ba, Ha Giang, Vietnam	AB769540
27	*Tylototriton* sp.	KUHE 55078	Quan Ba, Ha Giang, Vietnam	AB769541
28	*Tylototriton* sp.	VNMN 3389	Bao Lac, Cao Bang, Vietnam	AB769542
29	*Tylototriton* sp.	ROM 35330	Quang Thanh, Cao Bang, Vietnam	DQ517856
30	*Tylototriton* sp.	ROM 35364	Quang Thanh, Cao Bang, Vietnam	HM462056
Outgroup				
31	*T. kweichowensis*	KUHE 42282	Pet trade	AB769546
32	*T. taliangensis*	KUHE 43361	Pet trade	AB769543
33	*T. verrucosus*	KUHE 42348	Myanmar	AB769544
34	*Echinotriton andersoni*	KUHE no number	Nago, Okinawa, Japan	AB769545
35	*E. chinhaiensis*	MVZ 230536	Ningbo, Zhejiang, China	DQ517775
36	*Pleurodeles waltl*	MVZ 162384	Rabat, Morocco	DQ517813
37	*Notophthalmus viridescens*	MVZ 230959	St. Charles, Missouri, USA	DQ517795

mens collected near type localities of the remaining species of the group (Appendix 1). We identified specimens based on the original descriptions of each species of the *T. asperrimus* group. Voucher specimens examined are deposited at the Chengdu Institute of Biology, Chinese Academy of Sciences (CIB), the Graduate School of Human and Environmental Studies, Kyoto University (KUHE), Herpetological collection of the National Museum of Nature and Science, Tokyo (NSMT-H), and the Vietnam National Museum of Nature (VNMN).

The following 27 measurements were taken for morphological comparisons. Character definitions not mentioned below are given in Nishikawa et al. [2007, 2011]): SVL (snout-vent length) from tip of snout to anterior tip of vent; HL (head length); HW (head width); MXHW (maximum head width); SL (snout length); LJL (lower jaw length); ENL (eyelid-nostril length); IND (internarial distance); IOD (interorbital distance); UEW (upper eyelid width); UEL (upper eyelid length); OL (orbit length); AGD (axilla-groin distance); TRL (trunk length); TAL (tail length) from anterior tip of vent to tail tip; VL (vent length); BTAW (basal tail width); MTAW (medial tail width); BTAH (basal tail height); MXTAH (maximum tail height); MTAH (medial tail height); FLL (forelimb length); HLL (hindlimb length); 2FL (second finger length); 3FL (third finger length); 3TL (third toe length); and 5TL (fifth toe length). All measurements were taken to the nearest 0.1 mm with a dial caliper. We used a stereoscopic binocular microscope when necessary. The sex and maturity of the specimens were checked by minor dissections.

We compared SVL and 26 ratio values to SVL (R, %) among groups recognized in molecular analyses. Differences in SVL and ratio values were statistically tested among samples by Tukey-Kramer test and Kruskal-Wallis test with Dunn's multiple comparison test, respectively. The significance level was 95% in all these statistical tests.

We conducted multivariate analysis for examining overall morphological variation among groups recognized in molecular analyses. Using a total of 27 $\log_e$-transformed metric values, we conducted Principal Component Analysis (PCA) by SAS (1990).

We also examined skull morphology and counted the number of trunk vertebrae of specimens of *Tylototriton* sp. from Ha Giang (VNMN 3390) and from Cao Bang Provinces (VNMN 3389 and NSMT H5679–88). These characters were examined in soft X-ray photographs using Fuji Medical X-Ray Film.

RESULTS

Phylogenetic relationships and genetic divergence

We obtained 1035 bp sequences of partial ND2 region for 37 specimens, including outgroup (Table 1). Of 1035 nucleotide sites, 265 were variable and 244 were parsimony informative (sequence statistics available upon request from the senior author). The mean likelihood score of the Bayesian analyses for all trees sampled at stationarity was -6392.325. The likelihood value of the ML tree was -6343.875.

Phylogenetic analyses employing two different optimality criteria (BI and ML) yielded nearly identical topologies. We therefore present only the BI tree in Fig. 2. Monophyly of *Echinotriton* was fully supported in the BI and ML trees (bpp=100% and bs=97%), but that of *Tylototriton* was not supported in the BI tree (bpp=85% and bs=75%). Within *Tylototriton*, two major clades (Clades I and II) were recognized: Clade I consisted of species with orange or red markings on the body, and Clade II consisted of species with at blackish body (the *T. asperrimus* group). Each of these major clades was highly supported (I: bpp=100% and bs=99%; II: 100 and 81%).

Clade I included three species from China. Among them, *T. taliangensis* was first separated from a clade of *T. kweichowensis* and *T. verrucosus*.

Clade II was divided into four subclades: the

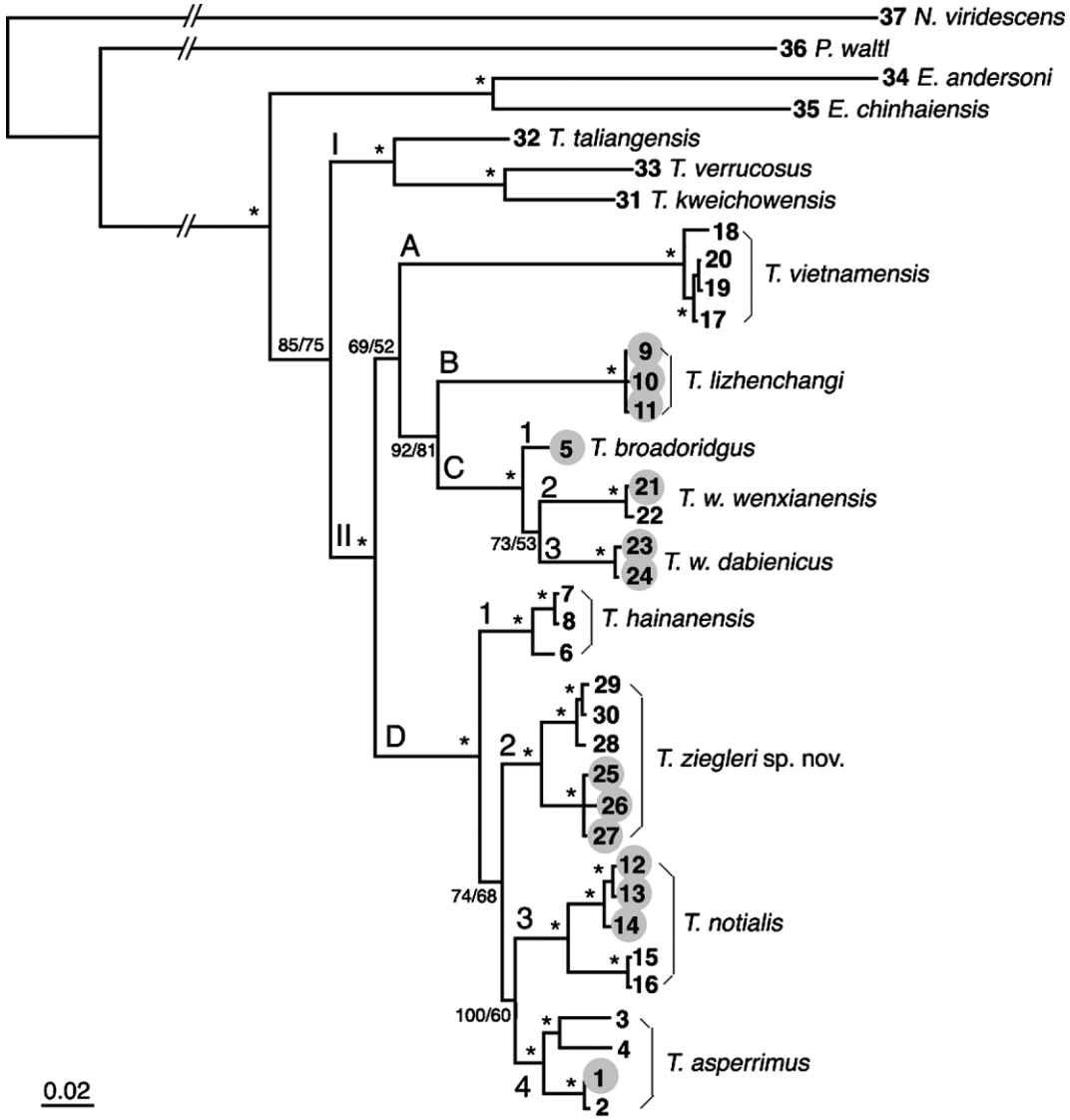

FIG. 2. Bayesian tree based on the partial ND2 gene for *Tylototriton* and related species (see Table 1 and Fig. 1). Numbers above branches represent Bayesian posterior probability and bootstrap supports for ML inference (bpp/bs). Asterisks indicate nodes with bpp≥95% and bs≥70%. Locality numbers shown in shaded circles indicate topotypic specimens of the species. Scale bar=0.02 substitutions/site.

subclade A of *T. vietnamensis* from eastern Vietnam (bpp=100 and bs=97%); the subclade B of *T. lizhenchangi* from southern Hunan, central China (100 and 97%); the subclade C of *T. broadoridgus* from northwestern Hunan, central China (C-1), *T. wenxianensis wenxianensis* from southern Gansu, northern China (C-2), and *T. w. dabienicus* from western Anhui, eastern China (C-3) (100 and 99%); the subclade D of *T. hainanensis* from Hainan, southern China (D-1), *Tylototriton* sp. from Ha Giang and Cao Bang, northern Vietnam (D-2), *T. notialis* from Khammouan, central Laos and from Nghe An, northern

TABLE 2. Mean and range (in parenthesis) of uncorrected p-distance between (below diagonal) and within (shaded in diagonal) nine groups recognized in molecular phylogeny.

	A	B	C1	C2	C3	D1	D2	D3	D4
A: *vietnamensis*	0.8 (0.1–1.2)								
B: *lizhenchangi*	11.8 (11.6–12.1)	— —							
C1: *broadoridgus*	10.5 (10.4–10.7)	7.7 —	— —						
C2: *w. wenxianensis*	10.6 (10.5–11.0)	8.1 —	3.8 (3.7–3.9)	0.2 —					
C3: *w. dabienicus*	10.9 (10.7–11.5)	8.8 (8.7–8.8)	3.3 (3.3–3.4)	4.9 (4.9–5.0)	0.1 —				
D1: *hainanensis*	10.7 (10.5–11.1)	9.0 (8.7–9.2)	7.5 (7.4–7.7)	8.9 (8.8–8.9)	8.9 (8.8–9.0)	1.3 —			
D2: sp.	11.3 (11.0–11.7)	10.0 (9.8–10.4)	8.0 (7.7–8.2)	9.4 (8.9–9.6)	9.3 (8.8–9.6)	4.4 (4.2–4.7)	1.7 (0.1–2.8)		
D3: *notialis*	11.4 (11.1–11.8)	9.9 (9.6–10.0)	7.9 (7.5–8.2)	8.9 (8.7–9.0)	9.1 (8.7–9.3)	4.7 (4.7–4.9)	5.0 (4.9–5.2)	1.9 (0.3–2.8)	
D4: *asperrimus*	11.0 (10.4–12.0)	10.3 (9.9–10.5)	7.7 (7.2–8.1)	9.0 (8.7–9.7)	9.2 (8.9–9.5)	5.0 (4.9–5.0)	4.7 (4.4–5.2)	4.8 (4.5–5.3)	2.7 (0.1–3.4)

Vietnam (D-3), and *T. asperrimus* from Guangxi, southern China and Hoa Binh, northern Vietnam (D-4) (100 and 81%). Divergence among the nine groups in Clade II (A, B, C-1, C-2, C-3, D-1, D-2, D-3, and D-4) was large but that within each group was small (Table 2).

The relationships among groups A to D, among C-1 to C-3, and among D-1 to D-4 were not constantly resolved by either analysis (Fig. 2). All of six averaged distances among groups D-1 to D-4 (4.4–5.0%) were greater than minimum values between other species in the *T. asperrimus* groups (≥3.3%: this study, Stuart et al., 2010, and Shen et al., 2012) and greater than or comparable to those between species of some Asian salamandrids (≥4.0% in *Paramesotriton*: Wu et al., 2010a; ≥6.6% in *Cynops*: Wu et al., 2010b).

In the subsequent morphological analyses, we compared differences among eight groups: *T. vietnamensis* (group A corresponding to the subclade A), *T. lizhenchangi* (B), *T. broadoridgus* (C-1), *T. wenxianensis* (C-2), *T. hainanensis* (D-1), *Tylototriton* sp. (D-2), *T. notialis* (D-3), and *T. asperrimus* (D-4), whose data were available from our original measurements and from the literature, with special attention to the morphological distinctness of *Tylototriton* sp. (D-2).

Morphological difference

A total of 40 adult males were used for comparison (Appendix 1). Values of SVL and ratios to SVL are shown in Tables 3 and 4, respectively.

Because of the paucity of specimens for statistical testing, we could examine statistical difference only among *Tylototriton* sp., *T. asperrimus*, *T. hainanensis*, and *T. vietnamensis*, of which we had more than three samples. In SVL, *Tylototriton* sp. was significantly larger than *T. hainanensis*. In the remaining measurements, *Tylototriton* sp. tended to show intermediate values among all of the species of the *T. asperrimus* group examined. In character ratios, *Tylototriton* sp. had significantly larger values than *T. asperri-*

TABLE 3. Measurements of specimens of the *Tylototriton asperrimus* group (mean±SD with range in parenthesis). Data of *T. broadoridgus* were taken from Shen et al. (2012). Note: SVL and TAL of *T. broadoridgus* probably include VL and exclude VL, respectively. H: holotype, P: paratype, T: topotype. M: male; F: female. For character abbreviations, refer to text.

Species	*Tylototriton* sp. (*ziegleri* sp. nov.)			*asperrimus*	*broado-ridgus*	*hainan-ensis*	*lizhen-changi*	*notialis*	*vietnam-ensis*	*wenxian-ensis*
Types	H	H, P	P	T	P		T			T
n and sex	M	19M	F	7M	29M	4M	2M	M	6M	M
SVL	68.0	61.9±3.8 (54.4–68.3)	70.8	59.8±2.5 (55.8–63.3)	66.5±3.2 (61.1–72.6)	56.2±0.6 (55.7–57.0)	59.9–63.8	61.5	58.0±1.3 (56.6–60.4)	61.2
HL	16.5	15.9±1.1 (13.5–17.9)	18.5	15.6±0.6 (15.0–16.8)	18.0±0.9 (16.0–20.2)	15.9±0.4 (15.3–16.2)	14.6–15.1	15.4	14.3±1.2 (12.3–15.6)	14.8
HW	17.2	15.5±1.3 (12.6–17.5)	18.2	15.4±0.7 (14.7–16.4)	—	15.8±0.3 (15.4–16.1)	13.4–14.9	15.7	14.9±0.8 (14.0–16.1)	14.7
MXHW	17.8	16.4±0.8 (15.2–18.1)	18.5	16.5±0.6 (15.8–17.4)	16.5±1.0 (15.0–18.4)	16.0±0.3 (15.6–16.3)	13.8–15.6	18.6	16.0±0.7 (15.1–17.0)	16.2
SL	7.6	6.8±0.5 (6.2–7.7)	7.1	6.9±0.2 (6.6–7.2)	—	6.4±0.4 (6.0–6.8)	6.1–7.2	6.8	6.7±0.5 (5.9–7.3)	6.2
LJL	14.2	11.7±1.1 (10.0–14.2)	12.1	10.7±0.6 (9.9–11.7)	—	10.8±0.3 (10.5–11.1)	9.9–10.7	11.4	11.1±0.8 (9.9–12.0)	10.5
ENL	4.4	4.3±0.3 (3.7–5.0)	4.3	4.5±0.3 (3.9–4.9)	—	4.4±0.4 (4.1–4.9)	3.7–4.0	3.9	4.4±0.3 (4.0–4.8)	4.2
IND	5.9	5.6±0.5 (4.8–6.7)	6.0	5.3±0.4 (4.8–5.9)	5.0±0.3 (4.5–5.5)	5.1±0.1 (4.9–5.2)	4.8–5.6	4.8	5.4±0.5 (4.7–6.0)	4.8
IOD	7.8	7.5±0.5 (6.2–8.1)	8.2	7.1±0.2 (6.7–7.4)	—	7.1±0.2 (6.8–7.3)	6.4–8.1	7.8	6.8±1.0 (6.0–8.4)	4.8
UEW	2.2	2.2±0.5 (1.6–3.8)	1.9	1.8±0.2 (1.5–2.0)	—	1.8±0.1 (1.6–1.9)	1.7–1.8	2.2	1.9±0.2 (1.7–2.1)	1.9
UEL	4.5	4.1±0.3 (3.6–4.7)	4.3	3.8±0.4 (3.1–4.2)	—	4.0±0.2 (3.8–4.2)	3.6–3.9	3.9	3.6±0.3 (3.4–4.1)	3.8
OL	3.0	2.9±0.2 (2.5–3.3)	2.9	2.0±0.1 (1.9–2.2)	—	2.8±0.4 (2.2–3.1)	2.2–2.4	2.7	2.5±0.2 (2.3–2.7)	2.0
AGD	34.6	30.4±2.8 (25.1–35.8)	37.3	31.3±2.0 (28.0–33.6)	—	25.9±2.7 (23.1–28.6)	31.0–32.7	30.6	28.7±1.4 (26.8–30.2)	27.5
TRL	51.5	46.0±3.2 (40.2–53.4)	52.3	44.2±2.1 (40.6–46.8)	—	40.3±0.6 (39.5–41.0)	45.3–48.7	46.1	43.6±1.5 (42.0–45.6)	46.4
TAL	63.5	65.2±5.4, n=18 (55.1–73.4)	71.4	62.0±4.0, n=6 (56.7–65.9)	59.8±4.4 (47.1–65.4)	65.1, n=1	49.3–74.4	58.5	59.1±4.9 (55.1–66.6)	61.7
VL	4.6	4.2±0.6 (3.1–4.9)	2.7	4.6±0.8 (3.8–5.8)	—	4.4±0.4 (4.1–4.9)	3.0–4.3	4.4	5.1±0.6 (4.1–5.8)	4.8
BTAW	6.1	5.6±0.9 (4.3–7.0)	6.3	6.7±0.4 (6.1–7.3)	8.6±0.5 (7.9–9.5)	6.1±0.1 (6.0–6.3)	5.0–5.4	6.4	5.2±1.3 (3.6–7.0)	8.2
MTAW	3.8	2.9±0.5 (2.1–3.8)	2.9	4.1±0.3 (3.8–4.6)	—	3.2±0.4, n=3 (2.9–3.6)	2.6–2.7	3.7	3.3±0.7 (2.5–4.1)	4.2
BTAH	7.1	7.4±0.8 (6.2–8.8)	7.0	8.7±0.6 (7.8–9.6)	—	7.6±0.4 (7.2–8.2)	5.8–6.6	8.7	7.2±1.4 (5.6–9.0)	8.8
MXTAH	7.7	7.7±0.9 (6.3–9.1)	7.1	9.7±0.9 (8.6–10.9)	10.8±0.9 (8.7–11.9)	8.6±0.6, n=3 (7.9–9.0)	6.0–6.9	9.0	7.6±2.0 (5.8–11.2)	9.2
MTAH	6.3	6.6±0.9 (5.4–8.5)	6.6	9.2±1.0 (8.1–10.9)	—	7.6±0.6, n=3 (7.1–8.2)	5.0–5.2	8.2	6.9±1.9 (4.9–10.1)	8.7
FLL	23.3	19.8±2.0 (16.5–23.5)	22.2	19.5±0.8 (18.4–20.6)	20.9±0.9 (19.7–22.4)	16.5±0.7 (15.6–17.2)	21.3–23.9	20.0	19.8±0.9 (18.3–21.0)	19.4
HLL	25.0	22.0±2.0 (18.7–25.0)	26.1	21.5±1.0 (19.8–22.6)	22.1±0.9 (2.02–23.7)	17.9±0.7 (17.3–18.9)	23.4–26.3	21.7	21.0±0.8 (20.1–22.1)	20.9
2FL	4.0	3.3±0.6 (2.1–4.0)	3.8	2.9±0.4 (2.4–3.6)	—	3.0±0.1 (2.9–3.1)	3.7–3.8	3.7	3.2±0.5 (2.6–3.8)	3.8
3FL	5.0	3.8±0.7 (2.9–5.3)	4.0	3.6±0.5 (2.9–4.2)	—	3.6±0.6 (3.1–4.2)	4.2–4.3	3.8	3.9±0.4 (3.6–4.6)	3.9
3TL	7.5	5.3±0.8 (4.2–7.5)	6.0	5.1±0.5 (4.2–5.8)	—	4.7±0.4 (4.3–5.1)	5.3–5.9	5.9	5.2±0.6 (4.4–6.0)	4.9
5TL	2.2	2.2±0.5 (1.2–3.2)	2.0	2.0±0.3 (1.5–2.3)	—	2.2±0.5 (1.8–2.9)	2.2–2.5	2.0	2.5±0.4 (2.1–3.0)	2.7

TABLE 4. Ratios of characters (R: %SVL) of the *Tylototriton asperrimus* group (median with range in parenthesis). Data of *T. broadoridgus* (probably average) were taken from Shen et al. (2012). Note: SVL and TAL of *T. broadoridgus* probably include VL and exclude VL, respectively. H: holotype, P: paratype, T: topotype. M: male; F: female. For character abbreviations, refer to text.

Species	*Tylototriton* sp. (*ziegleri* sp. nov.)			*asperrimus*	*broado-ridgus*	*hainanensis*	*lizhen-changi*	*notialis*	*vietnam-ensis*	*wenxian-ensis*
Types	H	H, P	P	T	P		T			T
n and sex	M	19M	F	7M	29M	4M	2M	M	6M	M
RHL	24.3	26.0 (21.8–27.5)	26.1	25.8 (25.0–27.2)	27.0	28.4 (27.4–29.1)	23.7–24.4	25.0	24.6 (21.4–26.9)	24.2
RHW	25.3	25.2 (22.8–27.0)	25.7	25.7 (25.2–26.3)	—	27.9 (27.6–28.9)	22.4–23.4	25.5	25.4 (24.4–26.8)	24.0
RMXHW	26.2	26.7 (24.2–28.1)	26.1	27.5 (26.7–28.5)	24.7	28.3 (27.9–29.3)	23.0–24.5	30.2	27.8 (26.4–28.6)	26.5
RSL	11.2	10.9 (10.0–12.0)	10.0	11.4 (10.7–12.2)	—	11.4 (10.7–12.3)	10.2–11.3	11.1	11.8 (10.3–12.1)	10.1
RLJL	20.9	19.1 (15.7–20.9)	17.1	17.5 (16.7–19.4)	—	19.3 (18.9–19.5)	16.8–16.8	18.5	19.9 (16.4–20.7)	17.2
RENL	6.5	6.9 (6.0–8.3)	6.1	7.6 (7.0–8.2)	—	7.8 (7.2–8.8)	6.2–6.3	6.3	7.6 (7.0–8.3)	6.9
RIND	8.7	9.1 (7.9–10.8)	8.5	8.8 (8.1–9.9)	7.5	8.9 (8.8–9.3)	8.0–8.8	7.8	9.5 (8.2–10.0)	7.8
RIOD	11.5	12.3 (10.4–13.2)	11.6	11.8 (11.3–12.5)	—	12.8 (12.2–13.0)	10.7–12.7	12.7	11.1 (10.5–13.9)	7.8
RUEW	3.2	3.4 (2.9–6.0)	2.7	3.0 (2.5–3.4)	—	3.2 (2.9–3.4)	2.7–3.0	3.6	3.2 (2.9–3.5)	3.1
RUEL	6.6	6.6 (5.6–7.8)	6.1	6.5 (5.2–6.6)	—	7.1 (6.8–7.5)	6.0–6.1	6.3	6.1 (5.9–6.8)	6.2
ROL	4.4	4.7 (3.8–5.3)	4.1	3.4 (3.2–3.7)	—	5.2 (3.9–5.4)	3.4–4.0	4.4	4.3 (4.0–4.5)	3.3
RAGD	50.9	49.3 (43.6–52.4)	52.7	52.4 (50.2–54.0)	—	45.8 (41.1–51.3)	51.3–51.8	49.8	49.9 (46.8–52.1)	44.9
RTRL	75.7	74.0 (72.5–78.2)	73.9	74.2 (72.8–75.0)	—	71.6 (70.9–72.6)	75.6–76.3	75.0	75.4 (73.1–78.6)	75.8
RTAL	93.4	106.4 [n=18] (92.9–118.6)	100.8	100.9 [n=6] (95.9–118.1)	90.0	116.7 [n=1]	82.3–116.6	95.1	99.8 (95.0–110.3)	100.8
RVL	6.8	7.1 (4.9–8.1)	3.8	7.0 (6.4–9.7)	—	7.8 (7.3–8.6)	4.7–7.2	7.2	9.0 (7.1–10.1)	7.8
RBTAW	9.0	9.3 (6.8–10.6)	8.9	11.5 (10.1–11.8)	13.0	10.9 (10.5–11.2)	7.8–9.0	10.4	9.0 (6.2–11.6)	13.4
RMTAW	5.6	4.6 (3.9–5.6)	4.1	6.7 (6.4–7.3)	—	5.6 [n=3] (5.1–6.4)	4.2–4.5	6.0	5.4 (4.3–7.1)	6.9
RBTAH	10.4	11.6 (10.4–14.1)	9.9	14.8 (13.2–15.9)	—	13.1 (12.9–14.6)	9.7–10.3	14.1	12.0 (9.7–15.2)	14.4
RMXTAH	11.3	12.2 (10.8–15.1)	10.0	16.7 (13.8–18.3)	16.3	15.6 [n=3] (14.2–16.0)	10.0–10.8	14.6	12.4 (10.0–18.5)	15.0
RMTAH	9.3	10.1 (9.1–14.1)	9.3	15.4 (13.1–18.3)	—	13.3 [n=3] (12.7–14.4)	7.8–8.7	13.3	11.1 (8.4–16.7)	14.2
RFLL	34.3	31.6 (28.7–35.6)	31.4	32.4 (30.6–34.6)	31.5	29.2 (28.0–30.8)	35.6–37.5	32.5	34.0 (31.9–36.2)	31.7
RHLL	36.8	35.4 (32.7–38.2)	36.9	35.8 (33.4–37.8)	33.2	31.7 (31.0–33.2)	39.1–41.2	35.3	36.6 (33.3–38.1)	34.2
R2FL	5.9	5.7 (3.3–6.5)	5.4	4.7 (4.0–6.1)	—	5.3 (5.2–5.4)	6.0–6.3	6.0	5.8 (4.5–6.6)	6.2
R3FL	7.4	6.1 (4.9–7.8)	5.6	5.8 (4.9–7.1)	—	6.4 (5.6–7.4)	6.6–7.2	6.2	6.4 (6.3–7.9)	6.4
R3TL	11.0	8.4 (7.3–11.0)	8.5	8.5 (7.1–9.8)	—	8.4 (7.7–9.1)	8.8–9.2	9.6	8.9 (7.7–10.6)	8.0
R5TL	3.2	3.6 (2.0–4.8)	2.8	3.4 (2.5–3.8)	—	3.5 (3.2–3.5)	3.4–4.2	3.3	4.2 (3.5–5.3)	4.4

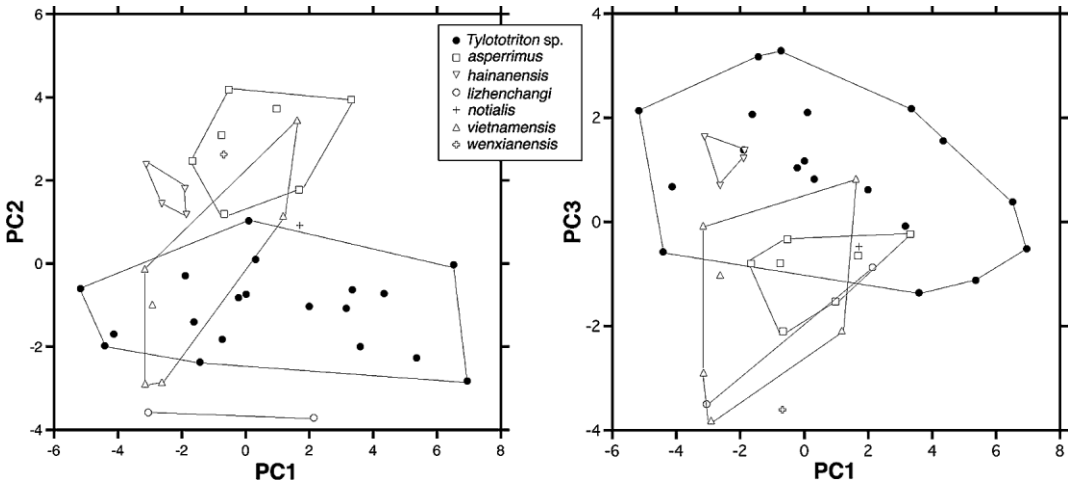

FIG. 3. Two dimensional plots of the second principal component (PC2) versus the first (PC1) (left) and the third (PC3) versus PC1 (right).

mus in ROL and *T. hainanensis* in RTRL and RHLL, and smaller values than *T. asperrimus* in RAGD, RBTAW, RMTAW, RBTAH, RMXTAH, and RMTAH, than *T. hainanensis* in RHL, RHW, and RMXHW, and than *T. vietnamensis* in RVL. *Tylototriton asperrimus* had significantly larger values in RAGD and RHLL but smaller values in ROL than *T. hainanensis*. *Tylototriton hainanensis* was significantly larger than *T. vietnamensis* in RHL and RUEL but smaller in RTRL, RFLL, and RHLL.

In PCA, the first three principal components accounted for 62.2% of the total variation. A two-dimensional plot of PC2 versus PC1 (Fig. 3, left) indicated that *Tylototriton* sp. overlapped all the remaining species in PC1 values but tended to be separated from them except for *T. vietnamensis* in PC2 values. In a plot of PC3 versus PC1 (Fig. 3, right), *Tylototriton* sp. overlapped most of the remaining species to various degrees, but still tended to be separated from them except for *T. hainanensis* and *T. notialis* in PC3 values.

Color pattern varied among species. Most of the species were of a dark brown or black ground color and had bright orange on rib nodules (dorsolateral glandular warts), finger and toe tips, parts of soles and palms, and vent continuing to the ventral ridge of the tail. However, *T. vietnamensis* had a yellowish brown or light gray ground color, and in *T. lizhenchangi* the posterior end of the parotoid was bright orange. This bright orange region was very variable in size among individuals.

Dorsal granules were most prominent and most dense in *Tylototriton* sp., finest and most sparse in *T. lizhenchangi* and *T. vietnamensis*, and intermediate between them in the remaining species. Ventral granules were more developed and isolated from each other in *T. wenxianensis* but less developed and arranged in transverse striations in the remaining species. Bony ridges on the head were more developed in *Tylototriton* sp. than in the remaining species. Rib nodules were most prominent and showed knob-like morphology in *Tylototriton* sp., and were least developed (nearly indistinct) in *T. lizhenchangi* and *T. wenxianensis*. The vertebral ridge was most tubercular in *Tylototriton* sp., and smoothest (and also widest) in *T. broadoridgus* and *T. wenxianensis*.

Based on the arguments mentioned above, *Tylototriton* sp. from northern Vietnam was distinguishable from the remaining species of the *T. asperrimus* group in both molecular and morphological properties. We therefore describe the species as new:

SYSTEMATICS

Tylototriton ziegleri sp. nov.

(Figs. 4, 5)

Tylototriton vietnamensis: Böhme et al., 2005, p. 217 (part); Weisrock et al., 2006, p. 372; Shen et al., 2012, p. 23.

Tylototriton cf. *vietnamensis*: Stuart et al., 2010, p. 21; Gu et al., 2012, p. 22.

Tylototriton asperrimus: Nguyen et al., 2009, p. 189; Yuan et al., 2011, p. 579.

Holotype

VNMN 3390, an adult male from Mt. Ta Boc, Ban Thang (Thang Village), Tung Vai Municipality, Quan Ba District, Ha Giang Province, Northern Vietnam, (23°03'25" N, 104°50'47" E, 1357 m asl), collected on 20 April 2012 by Huy Quang Nguyen.

Paratypes

A total of 18 males and one female: VNMN 1310–1316 and VNMN 907, eight males, from Bac Quang District, Ha Giang Province,

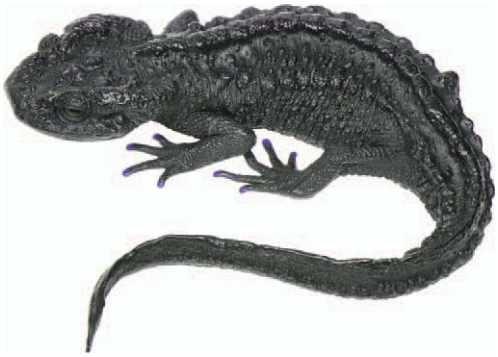

FIG. 4. The male holotype (VNMN 3390) of *Tylototriton ziegleri* in life.

FIG. 5. The male holotype (VNMN 3390) of *Tylototriton ziegleri*; dorsal (left) and ventral (right) views. Scale bar=20 mm.

Vietnam (22°21'50" N, 104°59'50" E, 885 m asl), collected in a bamboo forest on 12 May 2010 by Tao Thien Nguyen; VNMN 3389, one male from Bao Lac District, Cao Bang Province, Vietnam (1120 m asl), collected on 24 May 2012 by Dat Van Nguyen; NSMT H5679–5688, and nine males and one female from Mt. Pia Oac, Nguyen Binh District, Cao Bang Province, Vietnam (1250 m asl), collected on 24 May 1999 by S.-I. Uéno and A. Saito.

Etymology

The specific epithet is dedicated to Dr. Thomas Ziegler of Cologne Zoo (Germany), who has made great contributions to our understanding of the herpetofauna of Vietnam.

Diagnosis

A medium-sized newt of the genus *Tylototriton*, SVL 54.4–68.3 mm in males and 70.8 mm in the female; skin rough with fine granules; bony ridges on head distinct; vertebral ridge prominent and segmented, forming a row of tubercles; rib nodules prominent; limbs long and thin; tips of forelimbs and hindlimbs greatly overlapping when adpressed along body; tail thin; dorsum uniform dark brown or blackish; rib nodules, finger and toe tips, parts of soles and palms, and vent continuing to ventral ridge of tail bright orange.

Description of holotype

Body moderately stout; skin rough with fine granules, dense on dorsum and ventrum, but small and sparse on throat; head wider than long, hexagonal in shape, depressed and slightly oblique in profile; snout short, truncate, slightly beyond lower jaw; nostril close to snout tip; dorsolateral bony ridges on head prominent, from above eye to above anterior end of parotoid; middorsal ridge on head short, prominent; labial fold absent; skull wide and triangular, with dorsolateral and middorsal crests; maxillary connecting with quadrate, but slightly separated from pterygoid; frontsquamosal arch robust and wide; vomerine tooth series in an inverted V-shape, converging anteriorly but not reaching choana; tongue oval and attached to mouth floor but free laterally; parotoid distinct and projecting posteriorly; gular fold present, but weak; costal folds absent; vertebral ridge prominent and segmented, forming a row of tubercles, from neck to base of tail, separated from middorsal ridge on head with a small gap; rib nodules distinct, forming knob-like warts, 15 on right side and 16 on left side of body from axilla to base of tail; rib nodules increasing in size from most anterior to fourth nodule, then decreasing posteriorly; tips of fore- and hindlimbs greatly overlapping when adpressed along body (longest toe reaching at proximal end of palm); number of trunk vertebrae 13; fingers and toes free of webbing; tail laterally compressed, dorsal fin more distinct posteriorly, ventral ridge smooth, tip pointed.

Measurements of holotype (in mm)

SVL 68.0; HL 16.5; HW 17.2; MXHW 17.8; SL 7.6; LJL 14.2; ENL 4.4; IND 5.9; IOD 7.8; UEW 2.2; UEL 4.5; OL 3.0; AGD 34.6; TRL 51.5; TAL 63.5; VL 4.6; BTAW 6.1; MTAW 3.8; BTAH 7.1; MXTAH 7.7; MTAH 6.3; FLL 23.3; HLL 25.0; 2FL 4.0; 3FL 5.0; 3TL 7.5; 5TL 2.2; width of upper jaw tooth series 13.1; length of upper jaw tooth series 5.8; width of vomerine tooth series 4.3; length of vomerine tooth series 5.9.

Color

In life, dorsum uniformly black; venter slightly lighter than dorsum; rib nodules, finger and toe tips, parts of soles and palms, anterior end of vent, and ventral ridge of tail bright orange. In preservative, dorsal coloration tending to fade, becoming light brown and orange markings fading to cream.

Variation

A single female (SVL=70.8 mm) is larger than 19 males (54.4–68.3 mm). Males have more robust limbs than the female. The female has a relatively longer trunk (RAGD: 52.7%SVL in female vs. 43.6–52.4, median= 49.3%SVL in males), smaller tail height (RMXTAH: 10.0%SVL vs. 10.8–15.1, median=

12.2%SVL), and shorter vent slit than males (RVL: 3.8%SVL vs. 4.9–8.1, median=7.1%SVL). Male specimens of the type series are basically similar in morphology, but some specimens from one locality in Ha Giang and two localities in Cao Bang have less dense granules, less developed rib nodules, and smoother vertebral ridge than the holotype. Length of vomerine tooth rows is variable among specimens, from slightly exceeding to not reaching posterior end of choana. Number of trunk vertebrae varies slightly from 13 to 14 (usually 13). Specimens are generally constant in coloration, except for the absence or presence and extent of orange markings on palm and sole, and around vent.

Eggs

The mean diameter of ripe ova in ovaries that could be measured by a minor dissection of the paratype (NSMT H5679) ranged from 2.1 to 3.4 mm (n=17, gross mean=2.7 mm). The clutch size is unknown. The animal pole is dark brown and the remaining area is dark cream in color.

Larvae

Two larvae (KUHE 55077 and 55078 [Fig. 6]) were collected from a shallow pond (ca.10 cm in depth) in the type locality in Ha Giang Province on 20 April 2012 by Huy Quang Nguyen. Both of them were fully developed, but greatly differed in body size. The larger one (KUHE 55078) had a total length of 41.9 mm and the smaller one (KUHE 55077) had a total length of 23.9 mm. The following description of the larva is based on these two individuals.

Head nearly trapezoidal; depressed and sloping in profile; snout short and slightly truncate; labial fold distinct at posterior half of upper jaw; caudal fin higher than head; dorsal fin higher than ventral fin; origin of dorsal fin at middle of trunk; ventral fin originating from vent; tail tip rounded. In life, dorsum uniformly yellowish brown; venter whitish and transparent; golden spots scattered on dorsal head and trunk, lateral body, tail fin, and axilla to throat; fingers and toes yellow. In preservative, dorsal coloration tending to fade, becoming light brown, and golden spots fading to white.

FIG. 6. Larva (KUHE 55078) of *Tylototriton ziegleri*; dorsal (upper), lateral (middle), and ventral (bottom) views. Scale bar=10 mm.

Comparisons

Tylototriton ziegleri differs from all other species of the *T. asperrimus* group by having prominent bony ridges on head, denser granules on body, distinct tubercular vertebral ridge, and prominent knob-like rib nodules (vs. smoother skin, less developed ridges on head, slightly tubercular to smooth vertebral ridge, and slightly flattened to nearly indistinct rib nodules in *T. asperrimus*, *T. broadoridgus*, *T. hainanensis*, *T. lizhenchangi*, *T. notialis*, *T. vietnamensis*, and *T. wenxianensis*). The species further differs from *T. asperrimus* by having larger eyes and a thinner and lower tail; from *T. broadoridgus* by a narrower vertebral ridge, a relatively thinner and lower tail, and longer forelimbs and hindlimbs; from *T. hainanensis* by a larger body, relatively longer trunk and hindlimbs, and a shorter and narrower head; from *T. lizhenchangi* by a relatively longer and wider head, larger eyes, and shorter forelimbs and hindlimbs; from *T.*

notialis by a relatively narrower head (MXHW), and a thinner and lower tail; from *T. vietnamensis* by a relatively shorter vent slit, and a thinner and lower tail; and from *T. wenxianensis* by ventral granules arranged in transverse striations, a wider interorbital distance and larger eyes, and a thinner and lower tail.

Range

Ha Giang and Cao Bang provinces (probably also Lao Cai Province: see discussion below) in northern Vietnam.

Natural history

The species is basically terrestrial and very difficult to find except in the breeding season in April and May. The holotype was collected from a small permanent pond in a bamboo forest. The pond was 12 m long and 6 m wide, and water depth was about 15 cm, with a mud layer of ca. 30 cm on the bottom. The temperatures at the time of collection were air 23C, water 21C, and soil 21C. The humidity was 93% around the pond. The pH was lower than 6.5, carbonate hardness (KH) from 0 to 3, and general hardness (GH) lower than 3 in the pond. The adults were also found in temporal bodies of water that appear after rainfall, in small marshes in level areas, and in parts of overflowing streams. The eggs formed a mass on the ground near the pond, about 50–60 cm from the edge, without attending adults. Hatchlings seem to crawl to the pond on rainy days. Larvae metamorphose after July and do not overwinter.

DISCUSSION

Species delineation of the *T. asperrimus* group is practically very difficult, because each species of the group is very similar in body size, shape, and coloration (Stuart et al., 2010). *Tylototriton ziegleri* is here described as a new species in contrast to Yuan et al. (2011), who suggested that the species is a population of *T. asperrimus* based only on its relatively small genetic distance from the topotypic *T. asperrimus*. These authors also synonymized *T. hainanensis* and *T. notialis* with *T. asperrimus* on the basis of small genetic distance among them. As shown above, the genetic divergence between *T. ziegleri* and the remaining species of the *T. asperrimus* group is actually significant, when the distances among related species are considered. Moreover, morphological comparisons indicated that *T. ziegleri* is distinct from its congeners. In addition, we think that *T. asperrimus*, *T. hainanensis*, and *T. notialis* are also valid species based on the results of the present study.

Stuart et al. (2010) mentioned that the orange coloration on the posterior end of the parotoid was found only in *T. notialis* among species of the *T. asperrimus* group. However, this character is also present in *T. lizhenchangi* and some specimens of *T. asperrimus* and *T. wenxianensis* (Fei et al., 2010; Nishikawa et al., unpublished). In contrast, the specimens from Nghe An Province, northern Vietnam had no orange coloration on the parotoid although they were identified as *T. notialis* by the combination of other characters (see below). This identification is supported by our molecular results and those of Yuan et al. (2011) showing a very small genetic distance (2.8%: see Table 3) between the specimens from Nghe An and the type specimens of *T. notialis*. Thus, the presence of orange coloration on the parotoid can not be used as a diagnostic characteristic for *T. notialis*. Nevertheless, *T. notialis* can be distinguished by having a rougher dorsal skin than *T. lizhenchangi*, *T. vietnamensis*, and *T. wenxianensis*; by having a smoother skin than *T. ziegleri*; by larger eyes, and a thinner and shorter tail than *T. asperrimus*; and by having a shorter tail and longer limbs than *T. hainanensis* (see Table 4).

The known range of *T. ziegleri* is very limited. Nguyen et al. (2009) showed a photo of *T. asperrimus* from Lao Cai Province, northern Vietnam. The individual seems to be *T. ziegleri* based on its rough skin, tubercular vertebral ridge, and thin tail. Further field surveys in northern Vietnam and nearby

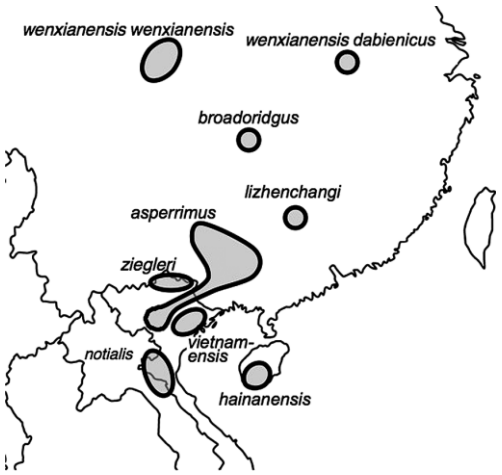

FIG. 7. Distribution of species of the *Tylototriton asperrimus* group. Populations unexamined in this study are not shown.

southern Yunnan Province, China are needed to clarify the actual distribution of *T. ziegleri*.

In the present study, the distribution of each species of the *T. asperrimus* group is presented in Fig. 7. A total of four species of the *T. asperrimus* group are currently known from northern Vietnam, namely *T. asperrimus*, *T. notialis*, *T. vietnamensis*, and *T. ziegleri*. According to Yuan et al. (2011), *T. hainanensis* is distributed in Baise, southern Guangxi, China, which is very close to Vietnam between the type locality of *T. asperrimus* (Sample number 1 in Table 1) and the locality of paratypic *T. ziegleri* (29 and 30). In addition, the record of *T. verrucosus* (the *T. verrucosus* group: Fei et al., 2005) from Lai Chau and Lao Cai provinces, northern Vietnam needs to be confirmed (Nguyen et al., 2009). There is no doubt that the northern Vietnam and its adjacent areas have the highest species diversity of *Tylototriton*. Until now, there has been no evidence of syntopic distribution of multiple species of *Tylototriton*. Northern Vietnam and its adjacent area where a maximum of six species are distributed is one of the best areas to study the ecological relationships such as species boundaries and difference in life history between species.

The species of *Tylototriton* are very popular as pets and are exported to Europe, North America, and Japan even though many species are protected in their native countries. Recent descriptions of several new species indicate that there is more cryptic diversity undiscovered in the genus (Shen et al., 2012; Hou et al., 2012; present study). Currently, habitat loss and degradation, especially around the breeding ponds, is a major threat to the populations of the new species. Legal protection of their habitats and regulation of excessive commercial collection are important measures for conservation of this species.

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Appendix 1

Specimens examined for morphometric and ratio comparisons

Tylototriton asperrimus: CIB GX200807010–16 (seven males) from Mt. Dayao, Jinxiu Yao Autonomous County, Guangxi Zhuang Autonomous Region, China (topotypes).

T. hainanensis: CIB 83360–63 (four males) from Jianfengling, Hainan Province, China.

T. lizhenchangi: KUHE 42316 and 42317 (two males) from Mt. Mang, Yizhang County, Hunan Province, China (topotypes).

T. notialis: VNMN 1194 (one male) from Pu Hoat District, Nghe An Province, Vietnam.

T. vietnamensis: KUHE 55172, VNMN 3032, 3168, 3363, 3364, 3374 (six males) from Yen Tu District, Bac Giang Province, Vietnam.

T. wenxianensis: CIB 750041 (one male) from Pingwu County, Sichuan Province, China.

T. ziegleri sp. nov.: VNMN 3390 from Quan Ba District, Ha Giang Province, Vietnam (one male), VNMN 1310–16, 907 (eight males) from Bac Quang District, Ha Giang Province, Vietnam, VNMN 3389 from Bao Lac District, Cao Bang Province, Vietnam (one male), NSMT-H05679–88 from Nguyen Binh District, Cao Bang Province, Vietnam (nine males and one female).

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